

Table 2: Peptide Binding to Bcl-2/iso1

Sequence	SEQ. ID NO:	K _d (nM)
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NLAAAQRYGRELRRMSDEFVDSFKK	23	39
AAAAAQRYGRELRRMSDEFVDSFKK	17	74
NLWGAQRYGRELRRMSDEFVDSFKK	24	159
NLWAGQRYGRELRRMSDEFVDSFKK	25	105
NLWAAQRYGRELRRMSDEFVDAFKK	26	26
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NLWAAQRYGRELRRMSDEFVDSFAK	28	123
NLWAAQRYGRELRRMSDEFVDSFKA	29	22
GGGAAQRYGRELRRMSDEFVDSFKK	30	63
NLPAAQRYGRELRRMSDEFVDSFKK	31	54
NLWAAQRYARELRRMSDEFVAAFKK	32	186
NLWAAQRYGREARRMSDEFVDSFKK	33	7483
NLWAAQRYGRELRRMSAEFVDSFKK	34	762
QRYGRELRRMSDEFVDSFKK	35	711
NLWAAQRYGRELRRMSDEFVD	36	2326

Table 3: Peptide Binding to Bcl-2/iso2

Sequence	SEQ. ID NO:	K _d (nM)
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As Tables 2 and 3 show, the mutant proteins of the invention can be used for identifying compounds which bind tightly to a Bcl-2 family member.

EXAMPLE 3: Structure Determination of Bcl-2/iso1 and Bcl-2/iso2 by NMR

NMR Spectroscopy: The structures of soluble Bcl-2/iso1 and Bcl-2/iso2 were determined by NMR spectroscopy (see Figure 3). All NMR experiments were acquired at 298 K on a Bruker DRX500, DRX600 or DRX800 NMR spectrometer. Backbone ¹H, ¹³C, and ¹⁵N resonance assignments were achieved with [¹⁵N,¹³C,(75%)²H] Bcl-2 using a suite of deuterium-decoupled, triple-resonance experiments (HNCA, HN(CO)CA, HN(CA)CB, HN(COCA)CB, HNCO and HN(CA)CO) (Yamazaki, T., Lee, W., Arrowsmith, C. H., Muhandiram, D. R. & Kay, L. E. (1994) *J. Am. Chem. Soc.* **116**, 11655-11666). The side-chain ¹H and ¹³C NMR signals were assigned from HCCH-TOCSY experiments (Clore, G. M. & Gronenborn, A. M. (1994) *Methods Enzymol* **239**, 349-63), and stereospecific assignments of the valine and leucine methyl groups were obtained from an analysis of the ¹³C-¹³C coupling patterns observed for biosynthetically directed, fractionally ¹³C-labeled Bcl-2 (Neri, D., Szyperski, T., Otting, G., Senn, H. & Wüthrich, K. (1989) *Biochemistry* **28**, 7510-7516). NOE distance restraints were obtained from three-dimensional ¹⁵N- and ¹³C-edited NOESY spectra (Fesik, S. W. & Zuiderweg, E. R. P. (1988) *J. Magn. Reson.* **78**, 588-593, Marion, D., Driscoll, P. C., Kay, L. E., Wingfield, P. T., Bax, A., Gronenborn, A. M. & Clore, G. M. (1989) *Biochemistry* **29**, 6150-6156) acquired with a mixing time of 80 ms. Slowly exchanging amide protons were identified in an ¹⁵N-HSQC spectrum recorded immediately after exchanging the protein into a buffer prepared with D₂O. Residual dipolar couplings (HN-N and C'-C^α) were measured using uncoupled versions of the HNCO experiment on [¹⁵N,¹³C,(75%)²H] Bcl-2 in the presence of 17 mg mL⁻¹ Pf1 phage (Tjandra, N. (1999) *Structure* **7**, R205-R211, Hansen, M. R., Mueller, L. & Pardi, A. (1998)

Nature Struc. Biol. **5**, 1065-1074, Clore, G. M., Starich, M. R. & Gronenborn, A. M. (1998) *J. Am. Chem. Soc.* **120**, 1-571-10572).

Structure Calculations: Bcl-2 structures were calculated using a simulated annealing protocol (Brunger, A. T. (1992) *X-PLOR Version 3.1*. (Yale University Press, New Haven and London) with the program CNX (MSI, San Diego). A square-well potential ($F_{\text{NOE}} = 50$ kcal mol⁻¹) was employed to constrain NOE-derived distances. Based on the cross peak intensities, NOE-derived distance restraints were given upper bounds of 3.0, 4.0, 5.0, or 6.0 Å. Torsion angle restraints ϕ, ψ were generated from analysis of N, C', C α , and H α chemical shifts using the TALOS program (Cornilescu, G., Delaglio, F. & Bax, A. (1999) *J. Biomol. NMR* **13**, 289-302). A force constant of 200 kcal mol⁻¹ rad⁻² was applied to all torsional restraints. Explicit hydrogen bonds were included in α -helices only for residues observed to have slowly exchanging amide protons. The program PROCHECK was employed to analyze the geometric quality of the calculated structures in the ensemble (Laskowski, R. A., MacArthur, M. W., Moss, D. S. & Thornton, J. M. (1993) *J. Appl. Cryst.* **26**, 283-291).

The present invention is illustrated by way of the foregoing description and examples. The foregoing description is intended as a non-limiting illustration, since many variations will become apparent to those skilled in the art in view thereof. It is intended that all such variations within the scope and spirit of the appended claims be embraced thereby.

Changes can be made to the composition, operation and arrangement of the method of the present invention described herein without departing from the concept and scope of the invention as defined in the following claims.

Any references referred to herein are incorporated by reference.

11
2

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